

**FLETCHER ALLEN HEALTH CARE
MCHV CAMPUS
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DEPARTMENT OF LABORATORY MEDICINE – Chemistry Division

VARIANT GLYCOSYLATED HEMOGLOBIN (Hgb A1c)

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REFERENCES:

1. Variant Hemoglobin A1c program; Biorad, April 1994
2. Variant Operation Manual; Biorad, December 1993

PRINCIPLE:

The Biorad Variant is a fully automated glycosylated hemoglobin analyzer that utilizes the principle of ion exchange high performance liquid chromatography (HPLC) for separation of Hemoglobin A1c. The separation of Hemoglobin A1c is performed rapidly and precisely without interference from Schiff base, lipemia or temperature fluctuations.

Prior to analysis, 5 µl of patient sample or control is diluted with 1 ml of hemolysis reagent (citrate solution, pH 5.0) to hemolyze the blood and remove Schiff base. Once diluted, samples are incubated at 18-28°C for 10 minutes and are then ready for analysis.

The analyzer uses two dual-piston pumps that deliver a programmed buffer gradient of increasing ionic strength to the system. Prepared samples are maintained at a constant 8°C +/- 2°C in the autosampler chamber until they are automatically injected into the analytical flow path. (After each injection, the autosampler is rinsed with wash solution to reduce carryover). The sample is then carried by the buffer through the cation exchange cartridge. The hemoglobin is separated based on the attraction of the hemoglobin to the column. The separated hemoglobin then passes through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured. Background noise is reduced by measuring at an additional wavelength of 690 nm. The system uses a built-in integrator to perform the reduction of raw data collected from each analysis. A calibrator of known A1c concentration is run with each run for adjustment of the calculation parameters for the determination of Hgb A1c. A chromatogram displaying the changes in absorbance over time is printed for each sample. A report identifying each peak detected, the relative percent of each peak and retention times accompanies each chromatogram.

CLINICAL SIGNIFICANCE:

This assay is used to measure the amount of glycosylated hemoglobin in the blood. This measurement helps in the management of diabetic patients. It gives an accurate index of the mean blood glucose concentration over the past 2 months. A single fasting blood glucose measurement only gives an indication of the patient's immediate past condition (hours) but may not represent the true status of blood glucose regulation.